REMARKS

Informalities

By the foregoing amendment, the specification has been amended to clarify the disclosure. The specification refers to the nucleic acid sequence of annexin V (SEQ ID NO:1) and to the amino acid sequence of annexin V (SEQ ID NO:3). In the sequence listing, both sequences were designated as originating from *Homo sapiens*, as intended. However, through an inadvertent clerical error, the sequences that were inserted were from a different organism (*Rattus norvegicus*). Both the nucleotide sequence and the amino acid sequence of human annexin V were known prior to the present invention and are set forth in their entirety in Funakoshi *et al.*, Biochemistry 26:8087-8092 (1987) ("Funakoshi *et al.*"), the disclosure of which was incorporated by reference in the present specification. Page 6, paragraph [0019]. Moreover, the specification repeatedly confirms that embodiments of the invention comprise *human* annexin. See, e.g., page 2, paragraph [0006] ("[a] recombinant human annexin").

A corrected computer readable sequence listing and paper copy is also being submitted herewith (copy enclosed). In this sequence listing, the inadvertently inserted sequences have been corrected to list the intended human sequences, as set forth in Funakoshi *et al.* No new matter has been added.

Figure 1 has been modified to correct minor errors and provide clarification. Minor calculation errors in base pair counts have been corrected. There are 960 base pairs (rather than 959) in the first annexin V sequence shown in Figure 1 and there are 969 base pairs (rather than 968) in the second annexin V sequence shown in Figure 1. Additionally, the line separating the second annexin V sequence has been moved to fall where indicated (i.e., to the right of the leading "xxx"). These changes are supported by the specification, e.g., Page 36, paragraph [0099] ("The exact sequences *prior to* and after each annexin V sequence are unknown and denoted as "x". * * * The start codon before *each* annexin V gene must therefore be removed and a strong stop for tight expression should be added at the terminus of the *second* annexin V gene.") (emphasis added). The length of the human annexin V sequence without the stop codon is provided in Funakoshi *et al.* (963 base pairs); with the 9 base pair strong stop codon shown in Figure 1 (969 base pairs).

Amendments to the Drawings

The attached sheet of drawings includes changes to Figure 1. This sheet replaces the original sheet including Figure 1.

Attachment: Replacement Sheets

Restriction Requirement

Applicant affirms its election to prosecute the invention of Group I, claims 1-10, drawn to a method of treating sickle-cell disease with an annexin protein coupled to at least one protein.

Amendments to Claims

In the interests of furthering the prosecution of this application, claims 2-5 have been cancelled without prejudice to the subject matter contained therein. Applicant reserves the right to pursue the cancelled subject matter in a continuing (or subsequent related) application.

Claims 1 and 6-10 have been amended and new claims 16-20 have been added. Support for the amended and new claims is provided below.

Claim 1 has been amended to specify that the "additional protein" is a second annexin protein. Support for amended claim 14 is found, for example, at page 3, paragraph [0007], page 36, paragraph [0099] and original claim 5. Claims 6-10 have been amended so there is proper antecedent basis with respect to claim 1.

New claim 16 depends from claim 1 and further specifies that the first annexin protein is human annexin protein. Support for new claim 16 is found, for example, at page 2, paragraph [0006] and page 13, paragraph [0040]. New claim 16 is patentable for the same reasons as amended claim 1.

New claim 17 depends from claim 16 and further specifies that the first annexin protein is coupled to said second annexin protein by a protein linker. Support for new claim 16 is found, for example, at pages 36-37, paragraph [0099], and original claim 10. New claim 17 is patentable for the same reasons as new claim 16.

New claim 18 depends from claim 16 and further specifies that the second annexin protein is also human annexin protein. Support for new claim 18 is found, for example, at page 2, paragraph [0006] and page 13, paragraph [0040]. New claim 18 is patentable for the same reasons as claim 16.

New claim 19 depends from claim 18 and further specifies that the first annexin protein is coupled to said second annexin protein by a protein linker. Support for new claim 19 is found, for example, at pages 36-37, paragraph [0099], and original claim 10. New claim 19 is patentable for the same reasons as new claim 18.

Original claim 10 was objected to because the annexin V construct of SEQ ID NO:6 was not found in the prior art and thus, claim 10 is allowable in independent from; appropriate correction was required. New claim 20 is original claim 10 rewritten in independent form, including the limitations of the base claim and all the intervening claims.

Claim Rejections – 35 USC § 103

1. Claims 1, 3, 4 and 6

The Examiner has rejected claims 1, 3, 4 and 6 under 35 USC § 103(a) as being unpatentable over Thorpe *et al.* (U.S. Patent No. 6,312,694) in view of Tait *et al.* (U.S. Patent No. 5,632,986), Stamatoyannapoulos (U.S. Patent No. 4,965,251) and Bertling *et al.* (DE 195 41 284 A1).

The Examiner states that Thorpe *et al.* teach annexin conjugates for use as antithrombotics. To the contrary, Thorpe *et al.* teaches use of the annexin conjugates to *induce*, rather than prevent, the obstruction of blood vessels. See col. 5, lines 21-44 (instructs use of "a coagulation-inducing amount, or a vessel-occluding amount" of the conjugate).

Thorpe *et al.* report that aminophospholipids (such as PS) are expressed on the surface of the vasculature that supplies tumors with blood. The conjugates described by Thrope *et al.* comprise a "therapeutic agent" attached to a "targeting agent" that binds to aminophospholipids. Because the goal of Thorpe *et al.* is to destroy the tumor, the "therapeutic agents" are toxins and *coagulants*. Thorpe *et al.* use annexin merely as a targeting agent; a means to deliver the therapeutic agent to tumor vasculature. Indeed, Thorpe *et al.* state that annexin could be substituted with an antibody or another ligand with an affinity for aminophospholipids. See e.g., col. 24, lines 22-23 ("Both antibody and nonantibody targeting agents may be used, including ... annexins and related ligands.").

Thorpe *et al.* do not teach or suggest that an annexin protein could serve as a "therapeutic agent." Nor do Thorpe *et al.* teach or suggest the use of any annexin conjugate as an antithrombotic. In fact, any antithrombotic activity of the conjugate described by Thorpe *et al.* would be undesirable as the conjugate of Thorpe *et al.* is intended to *induce* coagulation and occlude vessels.

The Examiner states that Thorpe *et al.* suggest the use of annexin conjugates for the treatment of sickle cell anemia by incorporating WO 97/17084 by reference and suggesting substituting the annexin V starting materials in WO 97/17084 with the annexin conjugates of Thorpe *et al.* To the contrary, Thorpe *et al.* make clear that WO 97/17084 is incorporated for "for the purpose of describing annexin starting materials *for preparing constructs of the present invention*" (col. 44, lines 27-29; emphasis added). There is no suggestion that the annexin conjugates of Thorpe *et al.* could be substituted for the annexin V starting materials used in WO 97/17084. One of skill in the art would not be motivated to use the conjugate of Thorpe *et al.*, which *induces* coagulation and occlude vessels, to treat sickle cell anemia as it would be expected to exacerbate, rather than alleviate, the condition.

The Examiner states that Tait *et al.* teach annexin V protein conjugates that are effective as antithrombotics and methods for the use of such conjugates to treating related disorders (although Tait *et al.* does not mention sickle cell anemia or any anemia). The conjugates disclosed in Tait *et al.* comprise a first compound that has an affinity for phospholipids and a second compound that is capable of *lysing thrombi*. Thus, for example, Tait *et al.* disclose a conjugate of annexin coupled to urokinase. The affinity of annexin for phospholipids is exploited to deliver the thrombolytic agent to the site of the thrombus. One of skill in the art would not be motivated by Tait *et al.* to use annexin as the "second compound" (thrombolytic) because annexin does not lyse thrombi.

Moreover, one of skill in the art would not be motivated to combine the teachings of Tait *et al.* with those of Thorpe *et al.* The conjugate of Thrope *et al.* is intended to induce coagulation and occlude vessels while the conjugate of Tait *et al.* is intended to lyse thrombi. See Thorpe *et al.*, col. 41, lines 26-36 ("Even more telling is the disclosure of [Tait *et al.*] which, in complete contrast to the present invention, proposes the use of annexin as a conjugate with compounds that lyse thrombi, or precursors of such thrombolytic compounds. The referenced combination of an aminophospholipid binding protein, annexin, with a lytic agent is, evidently, the opposite of the present invention, which concerns the combination of annexin ... with agents that induce thrombosis, either directly or indirectly.")

The Examiner states that Stamatoyannapoulos teaches that in sickle cell anemia, sickled erythrocytes lead to thrombosis during the crisis stage of the disease. This clinical feature of sickle-cell anemia is also disclosed in the specification of the present application. See pages 2-3, paragraph [0004]. The treatment disclosed in Samatoyannapoulos involves the "pulsed" administration of erythropoietin to a patient in order to increase F-reticulocyte formation. There is no teaching or suggestion in Samatoyannapoulos of a method for treating treating sickle-cell disease using a modified annexin protein, or in particular, a modified annexin protein comprising a first annexin protein coupled to a second annexin protein. It is not clear why one of skill in the art would be motivated to combine the disclosure of Stamatoyannapoulos with the disclosure of the other references. However, even if combined, the disclosures would not teach or suggest the present invention. The affinity of annexin for phosphatidylserine and the anticoagulant activity of annexin were known. However, a major problem associated with any therapeutic use of annexins is their short half-life in the circulation. See page 11, paragraph [0034]. Most of the annexin is rapidly lost into the urine. The present invention provides a method of treatment for sickle cell anemia that uses a modified annexin protein that is both effective and has a prolonged half-life. See page 2, paragraph [0006]. None of the prior art discloses such a modified annexin or such a method for treating sickle cell anemia.

Finally, the Examiner states that Bertling *et al.* teach the use of annexin V treatment of sickle cell anemia. Bertling *et al.* European counterpart of the WO 97/17084 application discussed above that was incorporated by reference in Thorpe *et al.* Bertling *et al.* discloses the use of annexin to block the phosphatidylserine-dependent phagocytosis of erythrocytes in sickle cell anemia. Thus, the treatment is directed to the "anemia" – the deficiency of red blood cells – rather than the vaso-occlusive complications. However, Bertling *et al.* does not teach use of a modified annexin comprising a first annexin protein coupled to a second annexin protein. Nor does Bertling *et al.* disclose administering a modified annexin protein of any kind that is both effective and has a prolonged half-life in the circulation.

Thus, none of the references, alone or together, teach or suggest a method for treating sickle-cell disease in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a modified annexin protein,

wherein said modified annexin protein comprises a first annexin protein coupled to a second annexin protein.

2. Claims 1, 2 and 4-9

The Examiner has rejected claims 1, 2 and 4-9 under 35 USC § 103(a) as being unpatentable over Kasina *et al.* (U.S. Patent No. 5,968,477) in view of Tait *et al.*, Stamatoyannapoulos and Bertling *et al.*

The Examiner states that Kasina *et al.* teach that annexins, including annexin V, naturally form multimers and specifically dimers, indicating that annexin V inherently couples. Kasina *et al.* states that annexin multimers may be used as a component of the radiolabeled conjugates disclosed therein. However, Kasina *et al.* do not teach or suggest that annexin V "naturally forms multimers" or that they inherently couple. Nor does Kasina *et al.* teach the therapeutic use of annexin conjugates as antithrombotics. Rather, Kasina *et al.* disclose radiolabeled conjugates and methods for their use in diagnostic imaging of vascular thrombi. The annexin component of the conjugate merely serves to deliver the radiolabel to the thrombi target sites.

As mentioned above, an advantage of the modified annexin proteins of the present invention is that they have a longer half-life in the circulation which makes them suitable for therapeutic use. By contrast, the radiolabeled annexin conjugates disclosed in Kasina *et al.* make the best diagnostic agents when they have a short half-life and are quickly eliminated from circulation. See, e.g., col. 10, lines 3-37. This reduces background noise and limits exposure of the subject to radiation. See, e.g., col. 9, line 54 - col. 10, line 2. Indeed, Kasina *et al.* states that the radiolabeled annexin dimers are removed from the blood at about the same rate as wild-type monomeric annexin V (col. 23, lines 65-67). Thus, even if they could be used as anticoagulants (and as discussed above, Kasina *et al.* does *not* disclose or suggest that they could), the annexin conjugates disclosed in Kasina *et al.* would have the same problem associated with their therapeutic use as monomeric wild-type monomeric annexin. Page 7, paragraph [0014].

The Examiner acknowledges that Kasina *et al.* does not teach the use of annexin dimers in the treatment of sickle cell anemia. However, the Examiner states that such teaching is provided by the same references cited above with respect to Thorpe *et al.* (i.e., Tait *et al.*, Stamatoyannapoulos and Bertling *et al.*). However, for the same reasons provided above with respect to Thorpe *et al.*, none of the references, alone or together, teach or suggest a method for

treating sickle-cell disease in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a modified annexin protein, wherein said modified annexin protein comprises a first annexin protein coupled to a second annexin protein.

Closing Remarks

Applicant believes that the pending claims are in condition for allowance. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-5117, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-5117.

Respectfully submitted,

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